Published in final edited form as:
Curr Biol. 2013 January 7; 23(1): 83–86. doi:10.1016/j.cub.2012.11.035.

Nematode-trapping fungi eavesdrop on nematode pheromones

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Summary

The recognition of molecular patterns associated with specific pathogens or food sources is fundamental to ecology and plays a major role in the evolution of predator-prey relationships [1]. Recent studies showed that nematodes produce an evolutionarily highly conserved family of small molecules, the ascarosides, which serve essential functions in regulating nematode development and behavior [2-4]. Here we show that nematophagous fungi, natural predators of soil-dwelling nematodes [5], can detect and respond to ascarosides. Nematophagous fungi use specialized trapping devices to catch and consume nematodes, and previous studies demonstrated that most fungal species do not produce traps constitutively but rather initiate trap-formation in response to their prey [6]. We found that ascarosides, which are constitutively secreted by many species of soil-dwelling nematodes, represent a conserved molecular pattern used by nematophagous fungi to detect prey and trigger trap formation. Ascaroside-induced morphogenesis is conserved in several closely related species of nematophagous fungi and occurs only under nutrient-deprived condition. Our results demonstrate that microbial predators eavesdrop on chemical communication among their metazoan prey to regulate morphogenesis, providing a striking example of predator-prey co-evolution. We anticipate that these findings will have broader implications for understanding other inter-kingdom interactions involving nematodes, which are found in almost any ecological niche on Earth.

Results and Discussion

Nematophagous fungi are carnivorous species that prey on or parasitize nematodes [5]. More than 200 such species from the Phyla Ascomycota, Basidiomycota and Zygomycota have been described and it is thought that their nematode-predacious lifestyles arose independently via convergent evolution [7, 8]. Many of these remarkable microorganisms develop specialized predatory devices (adhesive or non-adhesive traps) to capture, kill and

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consume nematodes (Figure 1A and movie S1). One striking feature of the nematode-trapping fungi is that they can detect the presence of prey. The majorities of nematophagous fungi produce very few traps constitutively, but form abundant traps in the presence of nematodes. Earlier work demonstrated that nematodes secrete a morphogenic substance that induces trap-formation [6]; however, the chemical composition of the trap-inducing factor remained elusive. From an evolutionary viewpoint, this trap-inducing factor likely represents a conserved, ancient marker of nematodes that must have played a critical role in nematode biology and promoted nematode fitness, outweighing the clear disadvantages of increased predation pressure.

Recent studies of the nematode *Caenorhabditis elegans* have identified a family of small molecules, ascarosides, as inter-organismal signals that play a central role in regulating nematode development and behavior [2-4]. The ascarosides are composed of the dideoxy-sugar ascarylose linked to a fatty acid-like side chain (Figure 1B and Figure S1), more than 100 different ascarosides have been identified from nematodes, and many function in synergy with overlapping activities [9]. For example, the ascarosides ascr#2 and ascr#5 are components of a pheromone that induces a larval diapause (dauer), whereas a blend of ascr#2, ascr#3, and ascr#8 function as a potent male attractant [10, 11]. Ascaroside biosynthesis and signaling are widely conserved even among phylogenetically distant nematode species, suggesting an ancient origin and conserved function for these signaling molecules [12]. Some ascarosides are broadly produced but interpreted differently by different species; for example, ascr#1 serves as a dauer-promoting signal in *C. elegans* but a male attractant in *Panagrellus redivivus*[13, 14]. Their wide conservation and essential roles in nematode biology suggested ascarosides as potential candidates for the nematode-derived cue that induces trap formation in nematophagous fungi. Therefore, we tested whether synthetic samples of ascarosides can induce trap morphogenesis.

Arthrobotrys oligospora is one of the most common and best understood species of nematophagous fungi that can be found in diverse soil environments. In the absence of nematodes *A. oligospora* persists as a saprophyte, whereas the presence of nematodes induces a shift to a predacious life style, producing a specific type of nematode trapping devices referred to as coil-like adhesive networks (AN), which are required for nematode-predation [15]. We confirmed that when cultured in the absence of nematodes on low-nutrient medium (LNM), *A. oligospora* produced very few traps, whereas adding nematodes to *A. oligospora* cultures dramatically induced trap-formation (Figure 1A). To investigate whether ascarosides affect trapinduction, we selected a set of 10 different ascarosides, including compounds that are widespread among many nematode species, e.g. ascr#1 and ascr#9, as well as compounds produced by relatively fewer species such as ascr#2 or ascr#8. A range of amounts of ascarosides was applied to *A. oligospora* mycelium cultured on LNM medium and the number of traps formed was counted after 48 hrs. We found that several of the tested ascarosides had strong trap-inducing activity, with ascarosides with 7- and 9-carbon side chains (ascr#1, 3, and 7) having the greatest effect (Figure 2A and B). Ascaroside-induced trap morphogenesis was concentration-dependent, requiring averaged concentrations as little as 0.5 nM to initiate trap-induction (Figure 3A). These ascaroside

concentrations are within the range of physiological concentrations observed in the vicinity of nematode population [16].

A. oligospora and many other nematophagous fungi of the Orbiliaceae are often found in decayed wood, where nitrogen is limited [17]. Predation of the nematodes provides nutrients, especially a nitrogen source, for *A. oligospora* under adverse growth conditions. It is known that trap-induction by nematodes requires nutrient-starvation [18]; we thus tested whether ascaroside-induced trap formation depends on the nutritional status of the fungus. As shown in Figure 3B, trap induction was completely suppressed in rich medium or LNM supplemented with nitrogen sources and significantly decreased with additional carbon source. These results suggest that nutrient-limitation, especially nitrogen starvation, is required for *A. oligospora* to sense and respond to ascarosides.

Next, we investigated whether ascaroside-sensing is specific to *A. oligospora* or conserved among nematode-trapping fungi. Ascaroside-responsiveness was assayed in seven additional species in the family Orbiliaceae, Ascomycota. Three of the tested species are phylogenetically closely related to *A. oligospora* with similar types of traps (AN), whereas the other four are more distantly-related and produce morphologically different traps (constricting rings or adhesive columns) (Figure 4). Under the conditions tested, ascaroside-induced trap formation was observed in all AN-producing species, but not in the other four species that form a separate clade (Figure 4). Intriguingly, the four AN-producing species responded to distinct but overlapping sets of ascarosides. For example, ascr#1, ascr#5, and ascr#9 were the strongest trap inducers for *A. musiformis*, whereas ascr#3, ascr#7, and ascr#9 were most potent in *A. javanica* (Figure 2A). Ascr#1, a strong trap-inducer in three tested species, did not induce a strong response in *A. javanica* (Figure 2A). It is possible that this species-specific variation of ascaroside-responsiveness is correlated to differences in ascaroside production of preferred or frequently encountered prey species. For the other four species that did not respond to ascarosides under the tested conditions (Figure 4), it is possible that they respond to other ascarosides not assayed here (there are more than 100 different nematode-produced ascarosides [9]) or non-ascaroside metabolites. Moreover, the fact that different ascaroside responsiveness was observed within just eight species examined also suggests that much specificity might exist among nematophagous fungi.

In their natural habitats, most organisms exist as part of complex multitrophic communities that depend on elaborate networks of chemical signaling. These inter-species interactions are critical to their survival and are often greatly shaped by evolution. In microbial communities, the molecular mechanisms of some interspecies interactions are well understood. For example, a quorum-sensing molecule, the autoinducer AI-2, mediates communication between different bacterial species [19], and bacterial peptidoglycan in the human serum can directly activate the adenylyl cyclase of the human fungal pathogen *Candida albicans* and triggers yeast-to-hypha transition [20]. Our finding that ascarosides trigger morphogenesis in nematophagous fungi demonstrates that evolutionarily conserved metazoan pheromones are perceived and interpreted by microorganisms. Ascarosides thus serve as nematode-associated molecular patterns that are recognized by fungi and perhaps other microorganisms, in analogy to the recognition of microbe-associated molecular patterns (MAMPs or PAMPs) by animals and plants [1, 21]. The inter-kingdom interactions

of nematodes and fungi we describe here further provide strong support for the hypothesis that predators often evolve to respond to essential prey communication [22].

Experimental Procedures

Trap induction assays

To test the role of ascarosides in trap induction, fungal cultures were grown on 20 ml of LNM medium at 25°C for 4 to 14 days until the diameter of the mycelium reached ~ 5 cm. 1 μl of 1 mM ascaroside solution or solvent control (25% EtOH) were dropped directly on the mycelium at the marked deposition sites (50 nM final concentration). After 48 hours, plates were observed under a dissecting microscope and the number of traps within 1.5 cm radius of the site of ascaroside drop deposition was quantified. Each species-chemical combination was tested independently 3-6 times. To test whether the addition of a carbon or nitrogen source affected ascaroside-induced trap formation, *A. oligospora* was grown on PDA and LNM medium supplemented with 2 % glucose or with 0.5 % ammonium sulfate. Trap-induction assays were then performed on *A. oligospora* grown on these supplemented media using ascr#7 as described above.

See also Supplemental Experimental Procedures.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank ATCC, CBS, and Dr. S. S. Tzean for providing fungal strains, A. Dillman for assisting with the construction of the phylogenetic tree, J. Leadbetter, H. Schwartz, M. Kato, J. Cho, S. von Reuss, and anonymous reviewers for valuable comments and suggestions. This work was supported by the National Institutes of Health (GM088290 to FCS) and the Howard Hughes Medical Institute, with which P.W.S. is an Investigator.

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Highlights

- Nematode pheromone ascarosides induce trap-formation in nematophagous fungi
- Ascaroside-induced fungal morphogenesis is conserved in several species
- Different nematophagous fungal species display distinct ascaroside response
- Ascarosides constitute a nematode-associated molecular pattern recognized by microbes

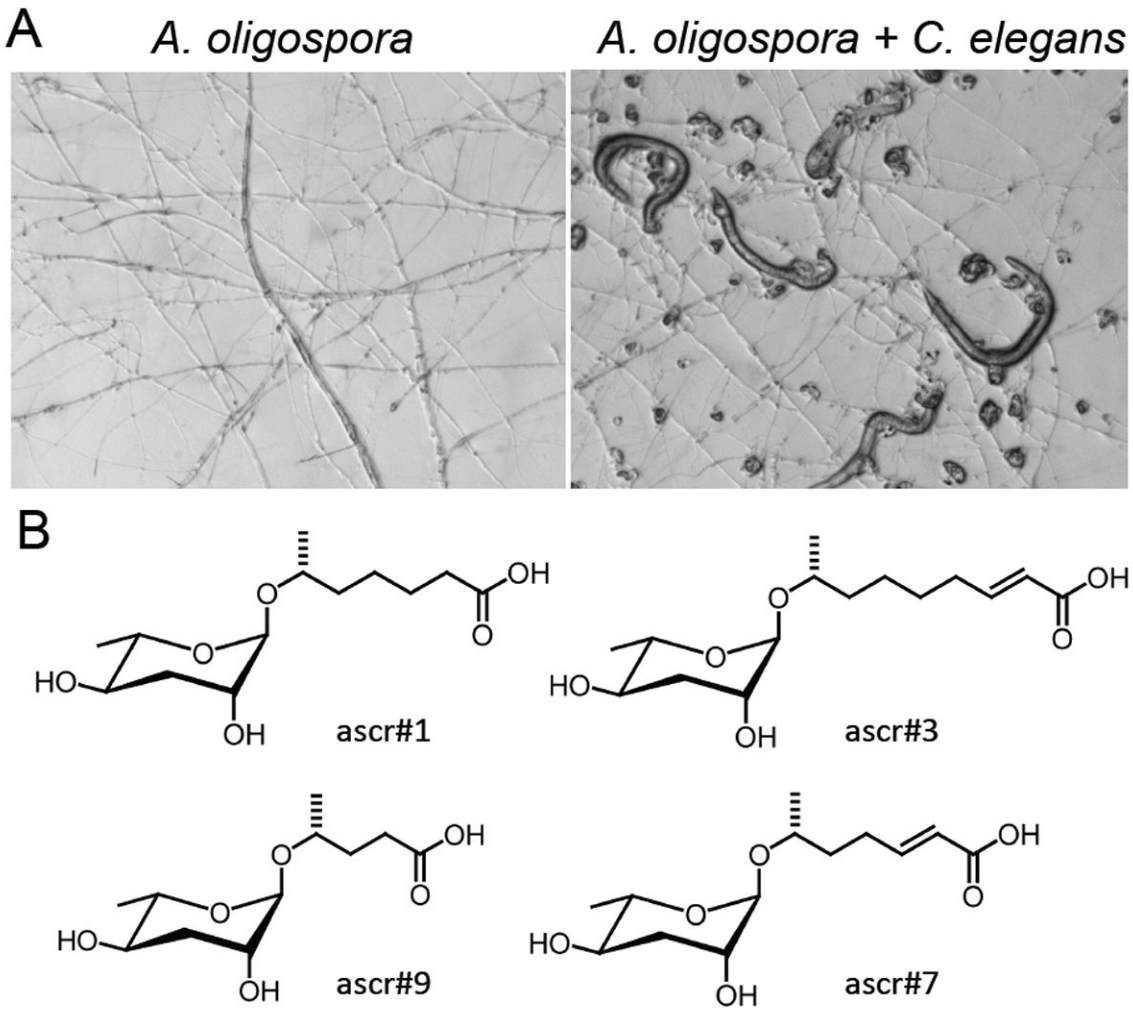

Figure 1.

Nematophagous fungi develop trapping structures in response to nematodes. (A) *A. oligospora* grown on LNM with or without *C. elegans*. (B) Chemical structures of ascarosides.

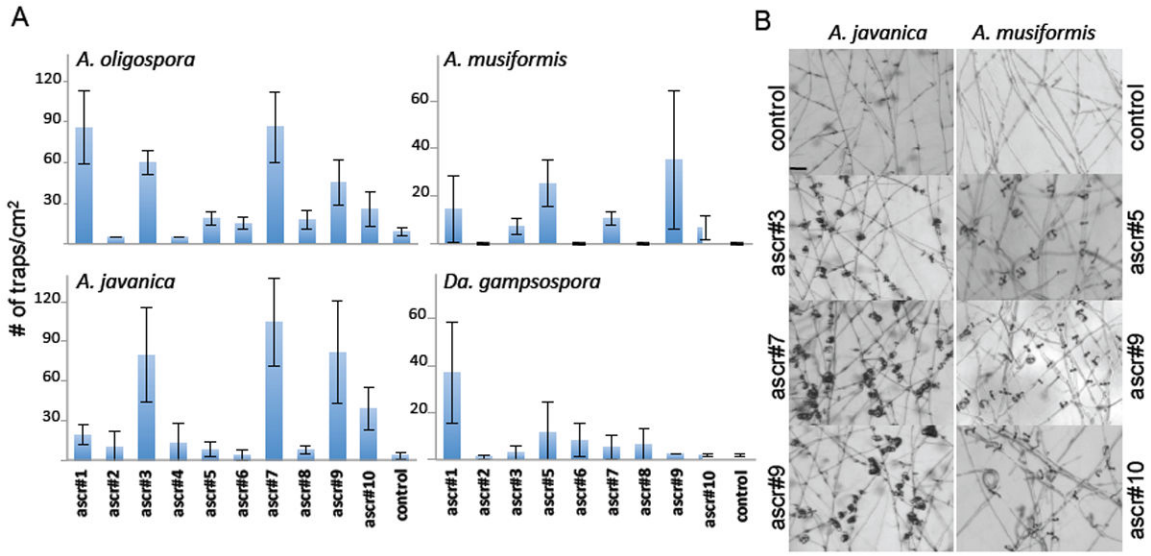

Figure 2.

Ascarosides induce trap-formation in nematophagous fungi. (A) Quantification of traps induced by 50 nM ascarosides or solvent control in *A. oligospora* and closely related species after 48 h (Mean ± SD, n = 3-4). *D.*, *Dactylella*. (B) Trap-induction of *A. javanica* and *A. misiformis* after 48 h of exposure to 50 nM ascarosides or solvent control. Scale bar indicates 100 μm.

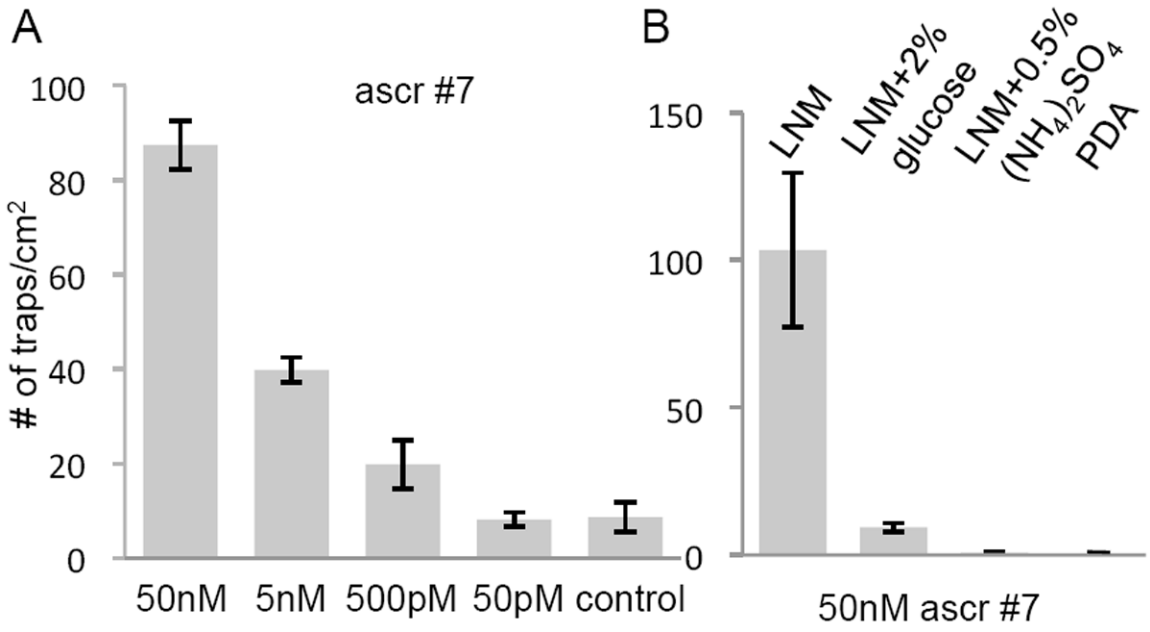

Figure 3.

Ascaroside-induced trap-formation is concentration-dependent and requires nutrient starvation. (A) Quantification of traps induced by indicated concentration in *A. oligospora* (Mean ± SD, n = 3). (B) Quantification of traps induced by 50 nM of ascr #7 in *A. oligospora* grown on different types of medium (Mean ± SD, n = 3). LNM, low nutrient medium; PDA, potato dextrose agar.

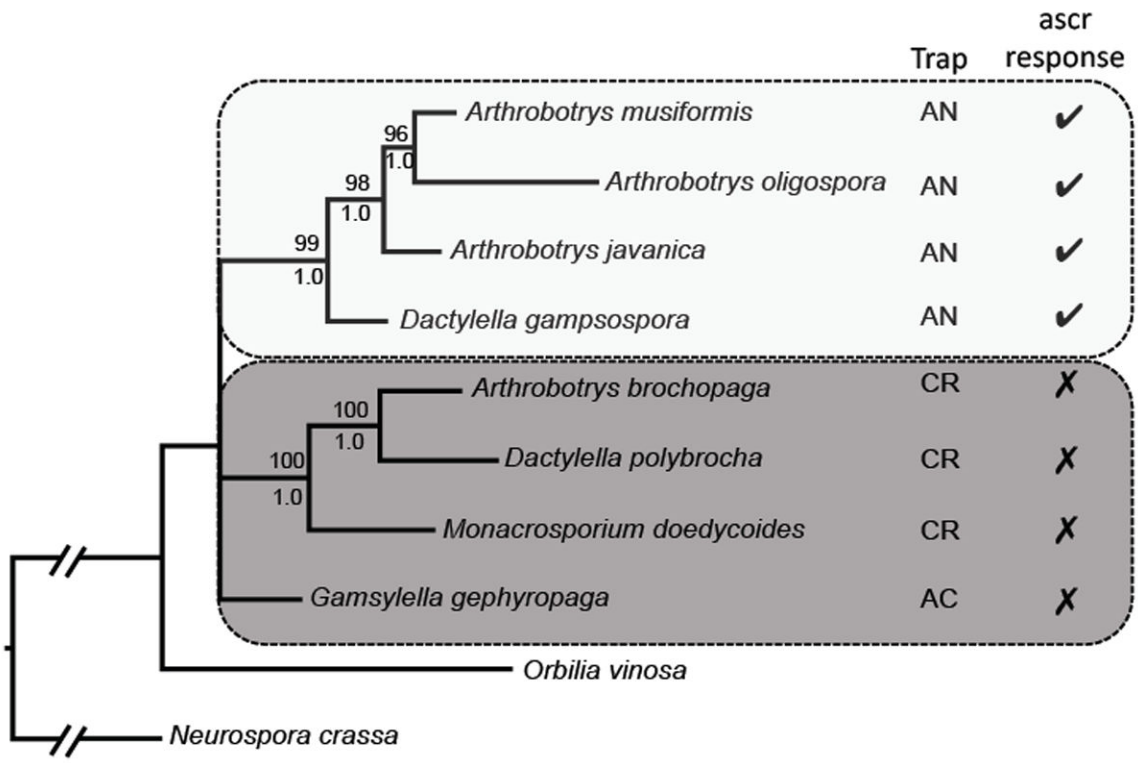

Figure 4.

Ascaroside-induced morphogenesis is conserved in *A. oligospora* and closely related species. Bayesian inference phylogeny of the ITS regions of eight nematophagous fungi in the family of Orbiliaceae. All are *Orbilia* species, but because the sexual stage has not been reported in many of these species, names referring to the conidial states are used. Numbers above and below the branches are maximum likelihood bootstrap value and posterior probabilities, respectively, where concordant. Only support values above 60% are shown. *Neurospora crassa* and *Orbilia vinosa* are outgroups. Trapping structures are AN, adhesive networks; CR, constricting rings; AC, adhesive columns.